III. REMARKS

Claim Status

Claims 14-21 and 27 are currently under examination and stand rejected.

Claim Objections

Claim 27 stands objected to under 37 CFR 1.75 as being a substantial duplicate of claim 14.

Applicant has cancelled claim 27 obviating this ground for objection.

Claim Rejections - 35 USC § 102

Claims 14-21 and 27 are rejected under 35 U.S.C. 102(a) as being anticipated by Yanagisawa et al. (International Immunology, 1997, Vol. 9 No. 2, pages 227-237) for essentially the reasons set forth in the previous office action in the rejection of claims 14-19, 21 and 27.

Applicant submits with this response, the Declaration of Dr. Florian Kern in support of its position that Yanagisawa et al. alone or in combination with Picker et al. does not anticipate or render obvious applicant's claimed invention.

Applicant believes this Declaration provides proper basis for applicant's position and defeats any *prima facie* case made out by the examiner.

In the interest of providing a complete integrated response, applicant herein repeats the background information provided in a prior response.

1. Incubation Time

In the instant application, the incubation time of T-cells with the protein fragment or fragments has been described as being "sufficiently long" to allow uptake of peptides into the binding groove of MHC molecules on one hand, and "sufficiently short" to avoid proliferation of T-cells stimulated by the presence of said peptides in the MHC binding groove.

Applicants have supplied citations confirming that anyone skilled in the set-up of cell culture experiments is aware that proliferation typically occurs not before 24 hours of stimulating T-cells.

Applicants would like to repeat that, as a statement of scientific fact, no proliferation can possibly occur within six hours and that this was current knowledge at the time of filing (and long before). Neither Applicants nor the examiner have provided any citations that teach otherwise. All provided citations support the well known fact that proliferation does not occur earlier than 24 hours after antigen-uptake, which is, indeed, one of the basic facts of human immunology.

Proliferation assays were among the earliest immunological assays performed and their basic kinetics had been established decades ago (publications from 1960's about this topic are available in PubMed).

> ....; and the incubation time of the suspension containing T-cells with the protein fragment or fragments is sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T-cells do not occur.

This original wording reflected two basic facts.

2. Specific elimination

Specific elimination of the T cells can occur through several mechanisms. Following successful activation of T-cells, a proportion of activated T-cells will naturally proliferate. Proliferation involves the synthesis of DNA, which will result in an increased uptake of nutrients, growth factors, and cytokines such as IL-2, required for cell survival.

In a limited culture space with a limited supply of said elements, T -cells that are more efficiently activated (T-cell clones with high avidity of the TCR for the NHC-peptide-complex) will have a proliferation advantage and exhibit higher consumption of nutrients, growth factors and cytokines. They will thus deprive other cells of these critical components.

By contrast, less efficiently activated T-cells (for example lower avidity clones) have a disadvantage and may not be able to take up sufficient amounts of nutrients, growth factors, and cytokines. The affinity/avidity model of T-cell activation was introduced in 1997 (Ward, ES, and Qadri, A, Current opinion in Immunology, 1997, 9:97-1061). In this sense, specific elimination occurs following specific activation, and the particular cells that are lost are those of lower affinity.

Because activation is specific (i.e. limited to those T-cells recognizing antigens like peptides presented in an MHC context) elimination of T-cells as a result of starvation following successful activation is equally specific. For example, if flu-specific peptides are used for stimulation, flu-specific T-cells may die in this way, but not tetanus specific T-cells. Using an incubation time of less than 24 hours, such effects are unlikely to occur. The shorter the incubation time, the less likely they are to occur.

3. Particular T-cells

"Particular" refers to those T-cells which, because of their lower avidity to the MHC-peptide complex will not be as strongly or effectively activated as T-cells of higher avidity to the WIC-peptide-complex.

The original wording was a reflection of these differences. In any case, "particular" is equivalent to "specific", meaning that these cells can be defined and are different by certain criteria than other cells in the culture. Even the fact of dying as such makes them particular, which, from applicants' point of view turns this discussion into a tautology. As soon as the cells die they are particular for that very reason, since the others do not. Applicants respectfully suggest that this is a semantic game, not a serious reason for rejecting a claim.

Applicants have observed that T-cells can require massive amounts of the survival factor IL-2. Unless IL-2 is added in sufficient amounts, T-cell responses (synthesis of cytokines) can be observed after 6 hours but not after 48 hours (proliferation) because activated cells are starved. That IL-2 needs to be added to T-cell cultures to prevent loss of cells during proliferation was already known in the early 90s. The addition of IL-2 for the culture of CMV specific T-cells stimulated by peptides was established long before the filing date of our application (already in 1991). Mark Wills in 1996 [Wills et al, 1996, J Virol, 70:7569-79, PDF supplied] used a limiting dilution proliferation assay to derive different clones from the stimulation by individual peptides. This illustrates further what applicants meant when they referred to specific elimination of particular cells.

In sum, applicants believe the language of the original claim and the language of the amended claim define the same

scope and are equivalent.

As stated by the examiner in the current office action, Applicant argues that since the time period specified by applicant in his method is 4 times shorter than the shortest time period specified by Yanagisawa et al. applicant has demonstrated a significant and useful difference between his invention and the process of Yanagisawa et al.

The examiner notes that the independent claims define the incubation time as a duration "sufficiently long so that the protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules said taking up being sufficient when an unambiguous identification of stimulated T cells is possible" and "... sufficiently short so that selection and proliferation accompanied by the specific elimination of stimulated T cells do not occur".

The examiner notes that this time period would necessarily vary from cell population to cell population. More importantly, the upper end of the claimed "range" is defined by the "the specific elimination of stimulated T cells" not occurring.

The examiner then concludes that since the cellular functions and surface markers of the stimulated cells are effectively measured by Yanagisawa et al., said cells have not be "specifically eliminated" and that Yanagisawa et al.'s method also requires that the incubation time of the protein fragment(s) with cell suspension containing T cells be of a duration "sufficiently long so that the protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules said taking up being sufficient when an unambiguous identification of stimulated T cells is possible" and "... sufficiently short so that selection and proliferation

accompanied by the specific elimination of stimulated T cells do not occur".

With regard to the limitation that the incubation time be sufficiently short so that selection, proliferation and the specific elimination of stimulated T cells does not occur, it is deemed, in absence to evidence to the contrary, that since the active expression of cell surface markers and are measured on the stimulated T cells, said cells could not have been specifically eliminated.

Applicant respectfully disagrees.

In addition to the remarks set forth in applicant's last response, repeated hereinabove for completeness of response applicant notes that Yanagisawa et al. (Int. Immunology, 1997, vol. 9, No. 2, pages 227-237 discloses a method wherein a suspension containing T-cells is examined after 4 days of incubation of the suspension with peptides derived from a protein originating from M. tuberculosis.

The composition with respect to T-cell receptor Vbeta chain usage is established by flow-cytometry.

By the time this is established, selection and proliferation of activated cells has occurred, which is an essential part of the method used by Yanagisawa et al.

Contrary to what is disclosed in Yanagisawa et al., applicant's method totally avoids selection and proliferation by the much shorter incubation period provided. This essential aspect of applicant's method is set forth in claim 1 and succeeding claims.

Yanagisawa et al. measures a cell composition with regard to T-cell receptor Vbeta family distribution. No activation

markers are measured by Yanagisawa.

Contrary to what is disclosed in Yanagisawa et al., the instantly claimed method measures activation markers of T-cells.

Furthermore, Yanagisawa et al. do not measure any effects at the single cell level whatsoever.

Based on the foregoing, the only conclusions that can properly be drawn from the disclosure of Yanagisawa et al. are:

1) proliferation of T-cells in response to a 96-hour long stimulation with peptides can be assessed by analyzing the composition of the cell suspension in regards of Vbeta family distribution;

2) stimulation with individual peptides can lead to a skewing of the distribution of T-cell receptor Vbeta family usage; and

3) Peptides leading to this skewing (Yanagisawa et al. were looking for an increase in the percentage of Vbeta 11+ CD4 T-cells) can be identified.

However, this allows the mapping of epitopes only at a rough population (Vbeta family) level, (Vbeta 11+ in this particular case) not at the single cell level.

Therefore, Yanagisawa et al. does not disclose the same method disclosed by applicant, selection and proliferation do occur in the method of Yanagisawa et al. and a *prima facie* case has not been made by the examiner. Applicant therefore respectfully requests favorable reconsideration of this ground for rejection.

Claim Rejections - 35 USC § 103

The rejection of claims 14-21 and 27 under 35 U.S.C. 103(a) as being unpatentable over Yanagisawa et al. (International Immunology, 1997, Vol. 9 No. 2, pages 227-237) and Picker et al. (Blood, 1995, Vol. 86 No. 4, pages 1408-1419) is maintained for reasons of record.

Picker et al. (Blood 86(4): 1408-1419) describes the use of flow-cytometry to identify intracellular cytokines following stimulation of cell suspensions containing T-cells with the super-antigens, SEA and SEB.

In this work Picker et al. did not use peptides for stimulation nor was the method used to identify epitopes.

The only appropriate conclusion that one can derive from the disclosure of Picker et al. is that activation of T-cells by super-antigens can be measured by flow cytometry at a single cell level if staining is performed intracellularly following retention of cytokines in the cell.

Applicant's claims are drawn to methods for identification of T-cell stimulating protein fragments comprising an incubation step where the incubation time of the protein fragment(s) with cell suspension containing T cells is of a duration "sufficiently long so that the protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules said taking up being sufficient when an unambiguous identification of stimulated T cells is possible" and "... sufficiently short so that selection and proliferation accompanied by the specific elimination of stimulated T cells do not occur".

As set forth above Yanagisawa et al. differs from the instant invention in that 1) the duration of the incubation is

sufficiently long so that selection and proliferation accompanied by the specific elimination of stimulated T cells does occur and 2) the cytokine levels in Yanagisawa et al. are measured by ELISA.

The examiner cites Picker et al. as disclosing a flow cytometric assay and concludes that it would have been obvious for one of ordinary skill in the art at the time of the invention to use the flow cytometry method of Picker et al. in the epitope mapping method of Yanagisawa et al. in order to take advantage rapid ability to determine the functional potential (i.e. response) of phenotypically distinct T cell subsets.

If one skilled in the art were to follow the examiner's suggestion, he would perhaps achieve an increase in the efficiency of the Yanagisawa et al. method but he would not have duplicated applicant's method which fundamentally differs from Yanagisawa et al. in the duration of the incubation. This significant difference in the duration of the required incubation period is one of the strongest indications of the difference between the instant method and the method resulting from the combination of Yanagisawa et al. and Picker et al.

Because the combination of references, even assuming *arguendo*, it were appropriate to combine them, discloses a method different from that claimed by applicant, applicant believes a prima facie case has not been made by the examiner and respectfully requests favorable reconsideration.

Conclusion

Applicant believes these remarks and the claim amendments are sufficient to obviate the grounds for rejection presented in the outstanding office action and respectfully requests

allowance of the pending claims. Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

Respectfully submitted,

NORRIS McLAUGHLIN & MARCUS, P.A.

By /Serle Ian Mosoff/

Serle Ian Mosoff
Attorney for Applicant(s)
Reg. No. 25,900
875 Third Avenue - 18th Floor
New York, New York 10022
Phone: (212) 808-0700
Fax: (212) 808-0844